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TITLE: TBI-Induced Formation of Toxic Tau and Its Biochemical Similarities to Tau in AD Brains

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13. SUPPLEMENTARY NOTES

14. ABSTRACT

The goal of the current study is to demonstrate that blast-induced traumatic brain injury (TBI) and Alzheimer's disease (AD) lead to similar biochemical changes in tau that increase its toxicity and contribute to cognitive and electrophysiological impairments. Specifically we will test the hypothesis that 1) blast-induced TBI leads to the production of a toxic form of tau that contributes to cognitive and electrophysiological impairments; 2) the formation of soluble tau aggregates contributes to cognitive impairments associated with both blast-exposure and AD; 3) an increase in tau phosphorylation contributes to cognitive impairments associated with both blast-exposure and AD. During the last year we have completed experiments related to the first point of the hypothesis, and started working on the second point. Specifically, we have found that the presence of tau is necessary for a preparation from shockwave-exposed mice to reduce 1) memory including contextual fear memory and spatial memory, and 2) long-term potentiation, a type of synaptic plasticity thought to underlie learning. We have also performed a dose response curve for the toxic effect of blasted tau onto memory and LTP.

15. SUBJECT TERMS
Contextual fear memory, spatial memory, synaptic plasticity, traumatic brain injury, Alzheimer's disease.

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1 INTRODUCTION

Although epidemiological studies find a strong link between TBI and an increased risk for dementia (i.e. AD), the molecular mechanisms responsible remain unclear. Evidence continues to accumulate highlighting the similarities between AD and post-TBI pathologies. A similarity between TBI and AD-related neurodegeneration exists at the histological level where both are characterized by the presence of aggregates of hyperphosphorylated forms of the microtubule associated protein, tau [1, 2]. Tau abnormalities and neurofibrillary tangles (NFTs), the classical histopathological hallmark of AD consisting of insoluble aggregated tau, have been reported in multiple animal models of TBI [3-6]. NFTs like those in AD have been reported after a single TBI in humans [7, 8]. Evidence also exists in favor of a link between TBI and amyloid-β (Aβ), the amyloid precursor protein (APP) proteolytic fragment thought to act upstream of tau in AD [9] that deposits in amyloid plaques. After experimental TBI in animal models, Aβ accumulated in injured neurons and axons both acutely [4, 5, 10, 11] and chronically [12]. Similar deposits of Aβ have been observed after a single TBI in humans [7, 8, 13]. The proposed research project seeks to define the toxic molecular mechanism leading to TBI and AD.

2 KEYWORDS

Tau, contextual fear memory, spatial memory, synaptic plasticity, traumatic brain injury, Alzheimer's disease

3 ACCOMPLISHMENTS

a. What were the major goals?

Work performed during the second year of funding aimed to complete experiments proposed in Aim 1 of the project "Test the hypothesis that blast induced TBI leads to the production of a toxic form of tau that contributes to cognitive and electrophysiological impairments". We also started to address experiments described in Aim 2 "Test the hypothesis that the formation of soluble tau aggregates contributes to cognitive impairments associated with both blast-exposure and AD". Specifically, we had to compare within month 16 the tau dependency of the behavioral and electrophysiological impairments caused by application of purified preparations in which tau has been excluded. Furthermore, we had to establish a dose response curve for the effects of tau derived from shockwave exposed mice onto memory and LTP. Finally, we had to identify the presence of tau in the brains of blasted mice similar to tau in the brains of AD patients.

b. What was accomplished under these goals?

To achieve the goal of demonstrating that changes occurring in tau following shockwave exposure are responsible for memory impairment we infused the extract of brain preparation normally used to isolate

tau onto the dorsal hippocampi bilaterally and tested behavior. Infusion of tau from blasted mice impaired both spatial memory and contextual fear memory compared with tau from sham-exposed mice. However, extracts from tau-KO mice (Jackson Lab (Stock #007251) did not impair both types of memory (Fig 1A-B). We obtained similar results when we examined LTP using tau preparation from C57Bl6 mice compared with a preparation extracted from tau-KO mice (Fig. 1C). These data suggest that shockwave-exposed tau undergoes changes capable of altering memory and synaptic plasticity. Interestingly, these results were similar to the effect of tau extracted with similar techniques from the brain of AD patients which impaired both memory and LTP (Fa et al, Sci Rep, 2016), suggesting the possibility that molecular similarities between tau prepared from shockwave-exposed mouse brains and human AD brains may underlie a common ability to produce cognitive impairment when infused into normal mice.

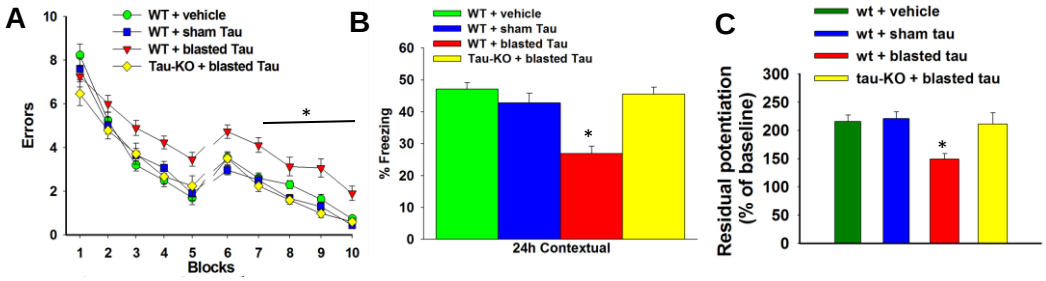

Fig. 1 Shockwave-exposure leads to the production of toxic tau in mouse brain impairing memory and LTP. A) 2-day RAWM performance in mice infused with 4.59 µg/ml tau purified from shockwave-exposed, tau-KO mice or sham animals (*p<0.05, RM-ANOVA, n= 7/10 animals per group). **B)** Percent of freezing during contextual FC test in mice infused with 4.59 µg/ml tau purified from shockwave-exposed, tau-KO or sham control animals (*p<0.05, Bonferroni post-hoc comparisons, n= 10/12 animals/group). **C)** Amounts of LTP in slices perfused for 20 min with 110 ng/ml shockwave exposed tau, extract from tau-KO or sham tau, prior to eliciting LTP (*p<0.05, Tukey's post-hoc test, n= 7/10 slices/group).

Next, we conducted analysis of the two-day radial arm water maze and contextual fear conditioning performance on animals infused with purified tau from either shockwave or sham-exposed mice at concentrations of 0.18, 0.92, 4.58 and 114.5 µg/ml. We have also compared the ability of tau to interfere with LTP when bath applied to acute hippocampal slice preparations at 0.92, 22.9, 110 and 573.5 ng/ml. We found a shift in the dose response curve between these two types of preparations.

Finally, we purified tau from the forebrains of mice subjected to shockwave or sham exposure 24 hrs prior to harvesting the brains as described previously. This method produces high quantities of protein (0.1-1.5mg tau from 1.5g of frozen tissue), preserves tau phosphorylation, and removes the vast majority of other proteins and DNA. When we analyzed the purified samples using non-reducing SDS-PAGE, we observed the presence of tau in the fractions obtained during chromatography (Fig. 2).

Interestingly, we obtained similar results when we used similar techniques to analyze tau extracted from human Alzheimer's brains (Fa et al, Sci Rep, 2016), conforming the possibility that molecular similarities between tau prepared from shockwave-exposed mouse brains and human AD brains may underlie a common ability to produce cognitive impairment when infused into normal mice.

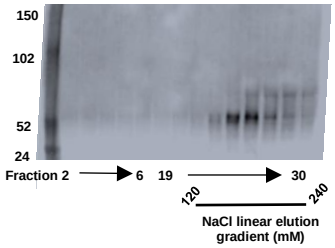

Fig. 2 Non-reducing SDS-PAGE analysis of murine tau from shockwave exposed animals homogenized in a non-reducing buffer, separated at different chromatographic conditions. Anti-tau immunoreactive bands are visible. Numbers at the bottom of the WB correspond to fraction samples obtained during chromatography. Brains were harvested 24 hrs after the blast.

c. What opportunities for training and professional development has the project provided

Nothing to Report"

d. How were the results disseminated to communities of interest?

Nothing to Report"

e. What do you plan to do during the next reporting period to accomplish the goals?

We will continue working on the hypothesis that blast induced TBI leads to the production of a toxic form of tau that contributes to cognitive and electrophysiological impairments. Specifically, we will continue investigating aim 2 "Test the hypothesis that the formation of soluble tau aggregates contributes to cognitive impairments associated with both blast-exposure and Alzheimer's disease" by examining whether tau oligomers are responsible for the impairment of memory and synaptic plasticity in shockwave-exposed mouse brains. We will also explore Aim 3 "Test the hypothesis that a similar increase in tau phosphorylation contributes to cognitive impairments associated with both blast-exposure and Alzheimer's Disease." by examining whether tau hyperphosphorylation is responsible for the impairment of memory and synaptic plasticity in shockwave-exposed mouse brains.

4 IMPACT

a. What was the impact on the development of the principal discipline

Our studies have provided depth to the identification of tau as a culprit in TBI.

b. What was the impact on other disciplines?

Our studies indicate a very interesting similarity between TBI and Alzheimer's disease with tau being similarly affected in the two conditions and being held responsible for the cognitive problems linked with them.

c. What was the impact on technology transfer?

Nothing to Report"

d. What was the impact on society beyond science and technology?

Our studies are important as they are likely to impact the development of therapies against TBI and Alzheimer's disease.

5 CHANGES/PROBLEMS

No changes, nor problems

6 PRODUCTS

Nothing to report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

a. What individuals have worked on the project

b. Name:	Ottavio Arancio
Project Role:	Principle Investigator
Researcher Identifier (e.g. ORCID ID):	1234567
Nearest person month worked:	1

Contribution to Project:	Dr. Arancio has supervised the whole project, insuring that the project is conducted in an efficient manner and with the highest scientific standards.
Funding Support:	National Institute of Health, Alzheimer's Drug Discovery Foundation

Name:	Russell Nicholls
Project Role:	Co-PI
Researcher Identifier (e.g. ORCID ID):	1234567
Nearest person month worked:	2
Contribution to Project:	Dr. Nicholls has provided his expertise on tau biochemistry and behavioral assessment. He has purified tau from sham and blasted mice.
Funding Support:	National Institute of Health

Name:	Barclay Morrison
Project Role:	Co-PI
Researcher Identifier (e.g. ORCID ID):	1234567
Nearest person month worked:	1
Contribution to Project:	Dr. Morrison has provided his expertise on traumatic brain injury and usage of blast tube.
Funding Support:	National Institute of Health, Paul Allen Family Foundation

Name:	Sowmya Sundaresh
Project Role:	Graduate Student

Researcher Identifier (e.g. ORCID ID):	1234567
Nearest person month worked:	6
Contribution to Project:	Sowmya has performed the behavioral experiments
Funding Support:	N/A

Name:	Lewis Brown
Project Role:	Co-Investigator
Researcher Identifier (e.g. ORCID ID):	1234567
Nearest person month worked:	1
Contribution to Project:	Coordinated Proteomic experiments
Funding Support:	

Name:	Nicholas Kanaan
Project Role:	Co-Investigator (Subaward PI)
Researcher Identifier (e.g. ORCID ID):	1234567
Nearest person month worked:	1
Contribution to Project:	Coordinated experiments, analyzed and interpreted data, and prepared reports.
Funding Support:	National Institute of Health, Brightfocus Foundation, Michael J. Fox Foundation

Name:	Collin Richards
Project Role:	Technician

Researcher Identifier (e.g. ORCID ID):	1234567
Nearest person month worked:	9
Contribution to Project:	Prepared samples, ran assays, collected and analyzed data
Funding Support:	N/A

Name:	Chelsea Hamel
Project Role:	Technician
Researcher Identifier (e.g. ORCID ID):	1234567
Nearest person month worked:	3
Contribution to Project:	Prepared samples, ran assays, collected and analyzed data
Funding Support:	N/A

c. Has there been a change in the active or other support of the PD or key personnel during the last reporting period?

Dr. Ottavio Arancio

W81XWH-15-1-0550 (Arancio) 09/15/2015 - 09/14/2018 1.20 Calendar
DoD
TBI-Induced Formation of Toxic Tau and Its Biochemical Similarities to Tau in AD Brains
This project seeks to determine changes in tau status that are evoked by traumatic brain injury.

R01 NS092045 (Arancio) 02/15/2015 - 12/31/2019 1.20 Calendar
NIH/NINDS
The regulation of beta-amyloid sensitivity and Alzheimer's related impairments by PP2A
This project seeks to examine the ability of the serine/threonine protein phosphatase, PP2A, to control sensitivity to the pathological actions of beta-amyloid, a protein that accumulates in the brain of Alzheimer’s disease patients.

R01 AG049402 (Arancio) 09/01/2015 - 03/31/2020 1.20 Calendar
NIH/NINDS
Extracellular tau oligomers and Alzheimer disease

This project seeks to establish extracellular soluble species of tau as major toxic species responsible for reduction of synaptic plasticity and memory in Alzheimer's disease.

ADDF 20160904 (Arancio) 10/15/2016 – 09/30/2017 0.12 Calendar
Alzheimer's Drug Discovery Foundation
A Novel Isoform Selective Kinase Inhibitor Candidate with In Vivo Efficacy in Two AD Models: Proposal for GLP Tox Package for Phase 1 IND
The goal of this project is to perform a toxicological study on compound MW150 in view of filing an IND with FDA

R01 NS067557 (Polleux) 03/15/2010 – 04/30/2021 0.76 Calendar
NIH/NINDS
Cellular and molecular mechanisms underlying the function of srgap2 during synaptic development
Test the roles of SRGAP2A and its human-specific gene copy in synaptic organization, circuit function and behavioral performance of cortical circuits.
Role: Co-Investigator

R01 AG050658 (Bartolini) 09/01/2016 – 05/31/2021 0.24 Calendar
NIH/NIA
Pathogenic role for formin mediated microtubule stabilization pathways in alzheimers disease
Test a unifying theory for the pathogenesis of Alzheimer's disease and examine the role for formins as potential targets in drug therapies aimed at rescuing Aβ and phospho--tau toxicity in Alzheimer's disease.

R01 AG050425 (Duff) 07/01/2015 - 06/30/2020 1.20 Calendar
NIH
Entorhinal-hippocampal circuit dysfunction in AD mice
Early stage Alzheimer's disease related cognitive impairment is thought to result from dysfunction in a circuit known as the entorhinal-cortex hippocampal circuit. To better understand the properties of cells in this circuit in an AD mouse model we will examine the firing properties of neurons, then use a computational model to predict how to restore function. We will then test the model by using optogenetics to stimulate neurons accordingly.

R56 (Arancio, Verderio) 04/01/2018 – 03/31/2023 2.04 calendar
NIH/NIA
On the role of microglia-derived extracellular vesicles in amyloid-beta induced changes in synaptic function and network activity in Alzheimer's disease
The goal of this project is to determine whether Aβ-containing extracellular vesicles originating from microglia may result in synaptic and network activity dysfunction in AD, and whether the cellular prion PrPc protein mediates trans-synaptic propagation of these vesicles.

Dr. Russell Nicholls

No Changes

Dr. Barclay Morrison

PI: Meaney/Morrison 07/2017 – 06/2022 1.92 calendar

Paul Allen Family Foundation 2,065,000
Reconstructing Concussion
The purpose of this grant is to uncover mechanisms and principles of concussive injury, repair and recovery at multiple scales from single cells to whole animals.

PI: Morrison 09/2017 – 12/2019 0.48 calendar
Department of Army
Long term potentiation deficits after repetitive primary blast
The purpose of this grant is to determine tolerance criteria to repetitive primary blast in organotypic brain slice cultures.

5R01EB009041 (Konofagou) 09/2014 – 08/2019 0.48 calendar
NIBIB
Optimization of ultrasound-facilitated blood-brain barrier opening
The purpose of this competitive renewal grant is to optimize ultrasound parameters for non-invasive opening of the BBB.

Dr. Lewis Brown

5R01MH098786-02 (Andrew J. Dwork, PI) 10/01/12—09/31/17 0.60 calendar
NIH
Building Schizophrenia Research in Macedonia
The major goals of our component of this project include performing the proteomic component of this work on human brain tissue and providing in-depth training for a research group in Macedonia as part of a foreign collaboration and research initiative.

NYSDOH C029159 Lewis Brown (PI) 4/1/14 –3/31/18 3.6 calendar
New York State Stem Cell Board
Grant Large-Scale Biochemical Profiling for Stem Cell Research in New York
This work involves metabolomic and proteomic studies of stem cells in New York.

1U01DA040582-01 Stavros Lomvardas (PI) 09/01/2015 – 08/31/2020 1.8 calendar
NIH
Tagging transcriptionally active olfactory receptor alleles and their activating enhancers towards the imaging and biochemical characterization of active olfactory receptor transcription.
This work will apply quantitative shotgun proteomics to identify and quantify proteins from affinity binding experiments with nucleome subcelllular structure from olfactory cells.

NSF IOS-1558030 (Kustka, Brown, Hildebrand: MPI) 01/01/2016 – 12/31/2017 (NCE) 1.2 calendar
NSF
IOS Collaborative Research: An integrated approach towards understanding iron uptake in marine eukaryotic phytoplankton
Work includes intensive profiling of the proteomes of Thalassiosira pseudonana and Phaeocystis globosa (grown under low and high Fe.

W81XWH-15-1-0550 (Arancio) 09/15/2015 - 09/14/2018 0.60 Calendar
DoD
TBI-Induced Formation of Toxic Tau and Its Biochemical Similarities to Tau in AD Brains
This project seeks to determine changes in tau status that are evoked by traumatic brain injury.

R56AI119854 Erich Mackow (PI) 04/01/2016-08/31/2019 0.36 Calendar
NIH
Novel Hantavirus Virulence Determinants
We will analyze proteins that bind to two tagged proteins in each year of the project.

Dr. Nicholas Kanaan

R01 AG044372 (Kanaan-PI) 9/30/14 – 4/30/19 3 months NIA
(NIH)
Tau-induced axonal degeneration in Alzheimer's disease and tauopathies
The main goal of this proposal is to identify the molecular mechanisms underlying axonal degeneration induced by AT8 phosphorylated tau using a viral vector rat model and a rat primary neuron model.

R01 NS082730 (Kanaan-PI, Brady-PI) 4/01/14-3/31/19 3 months
NINDS(NIH)
Tau Conformation in Tauopathies and Neuronal Function
This R01 is aimed at studying how tau conformation in various oligomeric forms affects its toxicity through axonal transport impairment and how tau conformation is regulated under normal biological conditions.

A2013364S (Kanaan-PI) 7/1/2013-6/30/2017 1.2 months
BrightFocus Foundation
Tau oligomers and their potential role in toxicity leading to Alzheimer's Disease
Goal: This project is aimed at further characterizing the structure and toxicity of tau oligomers generated under different experimental conditions (i.e. oxidative or reducing) and with various mutant forms of tau.

P01 AG14449 (Counts-PL, Kanaan-CoI) 7/01/97-6/30/19 1.2 months NIA
(NIH)
Neurobiology of Mild Cognitive Impairment in the Elderly
This PPG contains multiple projects that investigate the neurobiological substrates of cognitive decline in the elderly using the cholinergic basal forebrain as a model system for selective vulnerability.

AZ140095 (Arancio-PI; Kanaan-CoI) 9/1/15-8/30/2018 0.6 months
DoD
TBI-Induced Formation of Toxic Tau and Its Biochemical Similarities to Tau in AD Brains
The purpose of this grant is to explore the molecular mechanisms that underlie the cognitive decline and mental health problems resulting from repetitive traumatic brain injuries

146962 (Caryl Sortwell – PI, Kanaan Co-I) 0.6 months
Michael J. Fox Foundation 3/01/2016-2/28/2018

“Optimization of Nigrostriatal Degeneration in the Rat Alpha-Synuclein PFF Model”
Goal: This proposal will characterize the rat PD model induced by intrastriatal injection of preformed alpha-synuclein fibrils, with a focus on the neuropathological inclusions in this model.

d. What other organizations were involved as partners?

None to Report

8. SPECIAL REPORTING REQUIREMENTS

Not applicable

9. APPENDICES

Nothing to report